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Multifocal Electroretinograms in Normal Subjects

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Abstract: Multifocal electroretinography (ERG), developed by Sutter in 1992, is a method of recording the spatial distribution of focal ERG in a short time period using multi-input stimulation. Using this technique, we can detect the spatial extent and severity of damage to the macula. In this study, we recorded multifocal ERGs from 20 eyes of 20 normal subjects and analyzed the topographical properties of responses. In every subject, a negative wave followed by a positive wave could be recorded and we named them the N1-wave and the P1-wave, respectively. The amplitudes of the N1-wave and the P1-wave were the largest in the fovea, and they became smaller with eccentricity. In the P1-wave amplitude, the greatest intersubject variability was observed at the fovea. The N1 and P1 latencies were shorter in the upper retina than in the lower retina. The amplitude was larger in the upper retina than in the lower retina, which suggests the functional superiority of the upper retina. There was no statistical difference in latency and amplitude between the nasal and the temporal retina. We found no statistical difference between the responses of the papillomacular bundle and those of the temporal retinal area. The mapping obtained by multifocal ERG was useful as objective perimetry. **Jpn J Ophthalmol 1998;42:129–135** © 1998 Japanese Ophthalmological Society

Key Words: Functional superiority of upper retina, multifocal electroretinography, objective perimetry, topography.

Introduction

Multifocal electroretinography (ERG) developed by Sutter[1] utilizes the M-sequence method to map focal ERGs obtained from multiple retinal areas simultaneously in a short time period, and it shows them topographically. The amplitude of the response is reported to correspond to the density of cones and reflect the response of the outer layer of the retina.[1,2] It is possible to detect the spatial extent and severity of retinal impairment of the posterior pole, making this technique useful in clinical practice as an objective examination. Before clinical application, we recorded multifocal ERGs in normal subjects and analyzed the topographical properties of responses.

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Subjects and Methods

Subjects

Multifocal ERGs were recorded in 20 eyes of 20 normal subjects (9 men, 11 women) with no ocular disease except for refractive error. Subjects ranging in age from 21–76 years (mean = 29 years) were tested. The visual acuity was 1.0 or better with correction. Refractive error ranged from 0 to –7 diopters. Informed consent was obtained from each subject after a full explanation of the procedures.

Stimulation

The stimulus, consisting of 61 white/black hexagonal patterns, was shown on a 20-inch multiscan monitor (Flex Scan T660i-J, Nanao, Matsutou, Ishikawa, Japan) and was given within a central 25° field (Figure 1).

The luminance of the stimulus on the monitor was 127.90 cd/m^2 in the center of the white area, 5.87 cd/m^2 in the center of the black area, 100.01 cd/m^2 in the periphery of the white area, and 4.33 cd/m^2 in the periphery of the black area. The mean luminance was 60.38 cd/m^2. The contrast was set at 91.58%. The lu-

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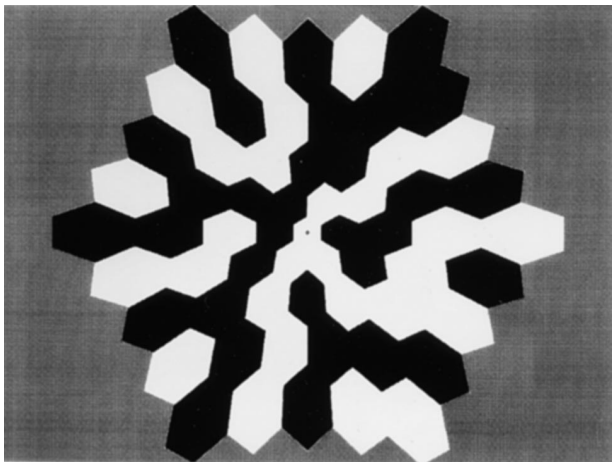

Figure 1. Stimulation monitor frame. The 61 white/black hexagonal patterns are arranged eccentrically. The subject is asked to fix his or her eye on a red fixation target at the center of the monitor.

minous intensity of the monitor around the stimulus pattern was 31.45 cd/m^2. Nine right eyes and 11 left eyes were examined; that is, the number of examined eyes was almost the same for the right and left so that asymmetry of the luminance of the monitor, if any, would be canceled. The stimulus consisted of an array of 61 hexagonal elements, with the element size scaled with eccentricity, each element being modulated white or black at the frequency of 75 Hz according to a binary M-sequence using on/off with a probability of 1/2. The area of hexagonal element was set so that the amplitudes of all focal ERGs were almost the same in normal subjects.[1] A small red fixation target was placed at the center of the stimulus.

Recording

The Burian-Allen contact lens electrode was used. After full dilation of the pupil by 0.5% tropicamide + 0.5% phenylephrine hydrochloride (Mydrin P), subjects were optically corrected to their best near visual acuities, when necessary. A ground electrode was attached to the forehead, and the fellow eye was occluded. Stimuli were provided by a television monitor placed 30 cm before the tested eye for 20–30 seconds, and one session of recording was carried out. We repeated this procedure 8–10 times with brief rest periods. Total recording time was 4 minutes. Signals were monitored on real time, and when there were artifacts due to ocular movement or eyelid movement, the responses were rejected. We used a chin rest to reduce these effects of the electromyogram.

Amplification of Electrical Signals and Response Analysis

Derived signals were amplified with the signal processor 7S12 (NEC-Sanei, Tokyo) and bandpass-filtered with high cut at 100 Hz and low cut at 5 Hz. The amplified signals were received in a personal computer (Macintosh Quadra 650, Apple, Cupertino, CA, USA) through an A/D converter and analyzed with software, Visual Evoked Response Imaging System 2.05 (VERIS 2.05) (EDI, San Francisco, CA, USA). Using cross-correlation analysis between the stimulation patterns and their responses, 61 focal ERGs were extracted. Trace array representation is a template in which the waveforms of the extracted 61 focal ERGs are displayed topographically, as are the visual fields (Figure 2). Responses can be grouped into more than one group. There are three types of averaging, as fol-

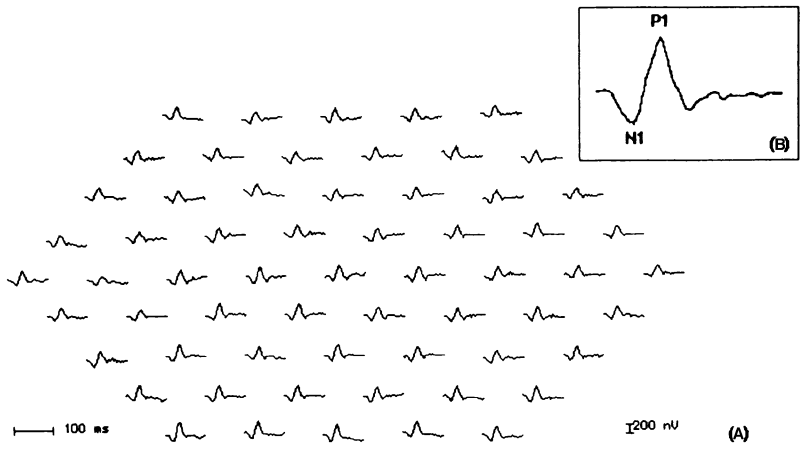

Figure 2. Trace array representation. (**A**) Representation of trace array from a normal left eye. The waves of 61 focal electroretinographies are topographically arranged. In every wave form, negative waves (N1-waves) and positive waves (P1-waves) can be seen as shown in (**B**).

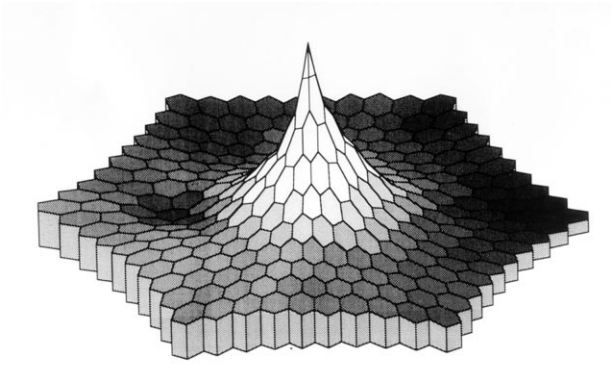

Figure 3. Three-dimensional representation extracted from a normal left eye shows a chevron topography with a peak in the fovea.

lows: (a) averages normalized (unit: nV), which represent the average of responses in each area; (b) averages, response density scaled (unit: nV/deg^2), which represent the amplitudes for each grouping adjusted for the distance angle of the stimulus element, giving a more accurate view of the actual response amplitudes of each group; (c) averages, sum of groups (unit: nV) are the amplitudes for the traces in each grouping added together. In the present article, we performed analyses using averages, response density scaled.

Three-dimensional (3D) representation shows 3D retinal topography (Figure 3). This is not the conventional noisy peak-to-peak measuring but is in accordance with the method of Sutter et al,[1,3] where one template wave or the averaging wave of 61 responses is multiplied by each focal response each time and divided by the hexagonal stimulating area to obtain the retinal response density. Representation by 3D shows a chevron topography with the macula as a peak in normal subjects and low columns in a retinal portion that has functional abnormality. This representation is visually easy to understand.

Analysis of Normal Response

In each wave of multifocal ERG, there is a negative wave followed by a positive wave like the a-wave and the b-wave in the ordinary ERG wave forms, but the intraretinal origins of these negative and positive waves remain obscure. We named them N1-wave and P1-wave, respectively (Figure 2). The amplitude of the N1-wave was measured from the baseline to the bottom of the N1-wave; that of the P1-wave was from the bottom of the N1-wave to the peak of the P1-wave. The peak latency was defined as the period from the time stimulation was given to the peak of each wave.

We grouped responses from all elements of the stimulus to learn the topographical properties of retinal responses as follows: (a) fovea and its outer portions, (b) upper and lower retina, (c) nasal and temporal retina, and (d) papillomacula bundle and the temporal portion corresponding to it. Comparison was made in each group.Responses from 20 eyes of 20 normal subjects were analyzed with averages, response density scaled, and the results were compared by repeated-measures analysis of variance for the fovea and its outer portions and by paired *t*-tests for the upper and lower retina, the nasal and temporal retina, and the papillomacula bundle and the temporal portion corresponding to it.

Results

Figure 2 indicates the trace array where the wave form in each retinal area is almost the same in size

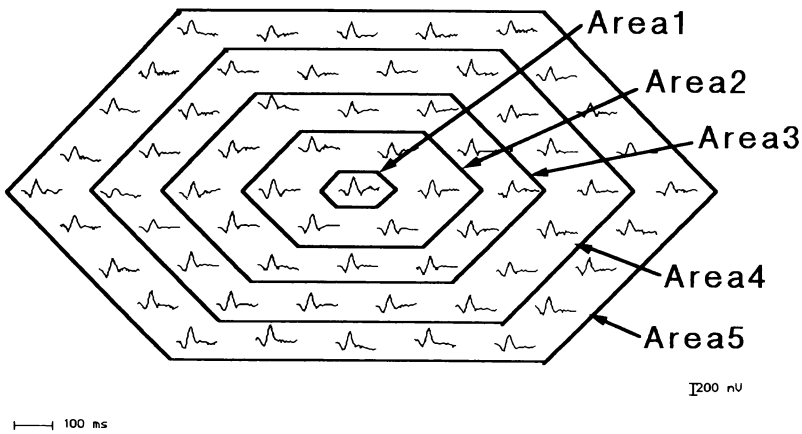

Figure 4. Comparison between fovea and its outer portions. Area 1 corresponds to the fovea, area 2 to the parafovea, and areas 3–5 correspond to the outer portions.

Jpn J Ophthalmol
Vol 42: 129–135, 1998

Table 1. Latency (msec) and Amplitude (nv) (Mean ± Standard Error) of the N- and P1-Waves at Five Different Stimulus Sites Illustrated in Figure 4

	Area 1	Area 2	Area 3	Area 4	Area 5
N1 latency	20.84 ± 0.35	20.50 ± 0.36	20.67 ± 0.31	20.75 ± 0.31	21.00 ± 0.33
N1 amplitude	16.37 ± 2.11	10.36 ± 1.15	6.30 ± 0.51	5.21 ± 0.55	4.49 ± 0.35
P1 latency	37.92 ± 0.29	37.08 ± 0.34	36.84 ± 0.29	37.00 ± 0.26	37.42 ± 0.31
P1 amplitude	46.16 ± 5.29	24.88 ± 2.25	16.50 ± 1.28	12.37 ± 1.01	11.08 ± 0.96

because of the size of the element. The response decreased in the portion including the optic disc in 18 of 20 eyes (90%). Three-dimensional representation is shown in Figure 3 with a central peak.

Comparison Between the Fovea and Its Outer Portions

We grouped responses in a trace array into five areas and named the areas 1 to 5, respectively, from the center to the periphery as the index of each wave form (Figure 4). Responses were totaled and averaged in each area. A negative wave (N1-wave) and a positive wave (P1-wave) were found in all areas. No statistical difference was found, but there was a tendency for N1 latency to be long at the fovea, shorter at the parafovea, and again longer at the perifovea ($P = 0.44$) (Table 1, Figure 5A). The P1 latency was long at the fovea, shorter at the parafovea, and again longer at the perifovea ($P < 0.0001$) (Figure 5C). The N1 and P1 amplitudes decreased from the fovea outward ($P < 0.0001$) (Figures 5B and 5D). The amplitude of the P1-wave showed the largest intersubject variation in the fovea (Figure 5D).

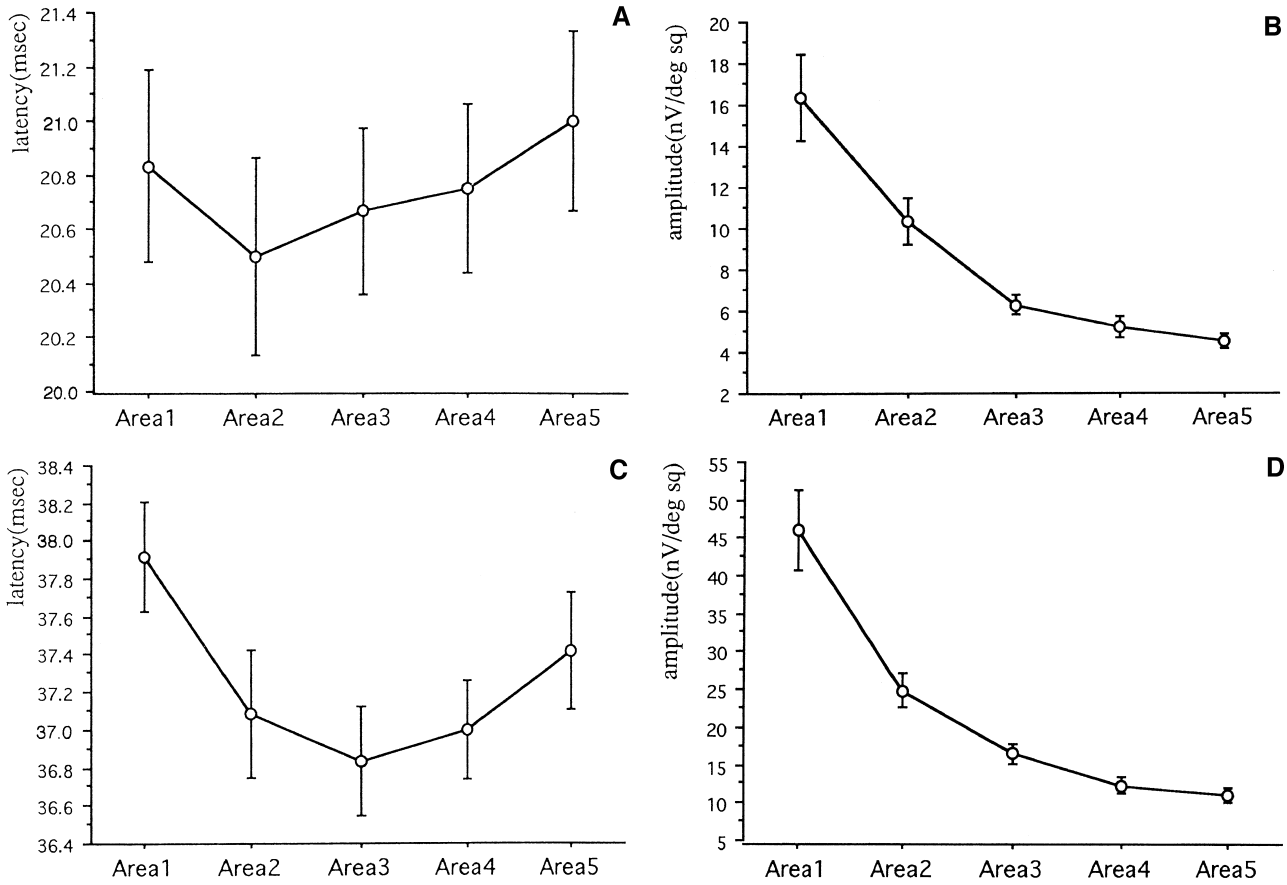

Figure 5. Comparison between fovea and its outer portions. Mean and standard error of N1 latency (**A**), N1 amplitude (**B**), P1 latency (**C**), and P1 amplitude (**D**) at five different stimulus sites illustrated in Figure 4.

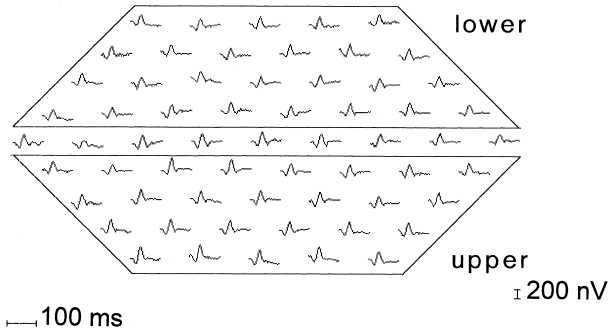

Figure 6. Comparison between the upper retina (lower half array) and lower retina (upper half array).

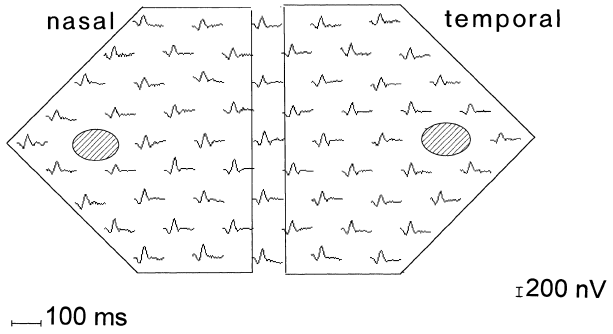

Figure 7. Comparison between the nasal and temporal retina. The portion that included the optic disc and the nasal portion corresponding to this oblique line portion was excluded from the summation.

Comparison Between the Upper and Lower Retina

We grouped each wave in the upper and lower retina as shown in Figure 6 and totaled and averaged focal ERGs in each group. The latency of the N1 wave was statistically shorter in the upper retina than in the lower retina (Table 2, $P = 0.02$). No statistical difference was found in N1 amplitude ($P = 0.31$) or P1 latency ($P = 0.58$). The P1 amplitude was statistically larger in the upper than in the lower retina ($P = 0.04$).

Comparison Between the Temporal and Nasal Retina

We grouped each wave in the nasal and temporal retina as shown in Figure 7 and totaled and averaged focal ERGs in each group. The portion including the optic disc was excluded from the summation. No statistical difference was found in N1 latency ($P = 0.75$), N1 amplitude ($P = 0.30$), P1 latency ($P = 0.08$), or P1 amplitude ($P = 0.45$) as shown in Table 3.

Comparison Between the Papillomacular Bundle and the Corresponding Temporal Portion

As shown in Figure 8, area 6 corresponds to the papillomacular bundle and area 7 to the temporal portion. We totaled and averaged focal ERGs in each portion. No statistical difference was found in N1 latency ($P = 0.42$), N1 amplitude ($P = 0.06$), P1 latency ($P = 0.10$), or P1 amplitude ($P = 0.77$) as shown in Table 4.

Discussion

The standard properties of the multifocal ERG topography in each retinal area as obtained in our results from 20 normal subjects would be important as basic data to evaluate measurements in eyes with pathology. Our study serves as a preliminary assessment of the potential clinical utility of this technique. We compared the responses in each retinal area to learn the topographic properties of retinal responses in normal subjects.

The amplitudes of N1-waves and P1-waves were the largest in the fovea, and they decreased with eccentricity. As Sutter et al[1] described before, this property of the response topography of multifocal ERG agreed well with the cone density distribution obtained by Curcio et al[4] from cadaver retinas. Although the luminance of the monitor also decreased outward, the decrease of the amplitude in area 5 compared to area 1 was 27%, while the decrease of the luminance of the monitor was within 73%. Therefore, the decrease of the amplitude would not

Table 2. Latency (msec) and Amplitude (nv) (Mean ± Standard Error) of the N- and P1-Waves at the Upper and Lower Retina

	Upper Retina	Lower Retina
N1 latency	20.25 ± 0.33	20.92 ± 0.33
N1 amplitude	5.81 ± 0.43	5.49 ± 0.54
P1 latency	37.00 ± 0.33	36.92 ± 0.30
P1 amplitude	14.37 ± 1.14	13.11 ± 1.11

Table 3. Latency (msec) and Amplitude (nv) (Mean ± Standard Error) of the N- and P1-Waves at the Temporal and Nasal Retina

	Temporal Retina	Nasal Retina
N1 latency	20.67 ± 0.35	20.58 ± 0.30
N1 amplitude	5.85 ± 0.57	5.55 ± 0.44
P1 latency	36.83 ± 0.27	37.08 ± 0.32
P1 amplitude	13.88 ± 1.21	14.14 ± 1.12

Jpn J Ophthalmol
Vol 42: 129–135, 1998

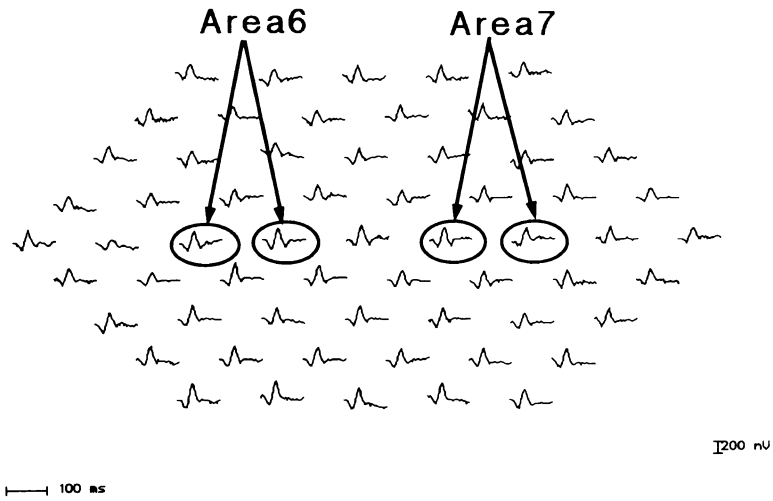

Figure 8. Comparison between the papillomacular bundle area and the area symmetrical to it (areas 6 and 7).

be dependent on only the luminance change of the monitor. The interindividual variation of the P1 amplitude was greatest in the fovea, as already reported by Sutter et al.[1] Histologically, Curcio et al[4] reported that the cone density of the human eye showed the greatest interindividual variation of the fovea in four eyes examined by them and in one eye examined by Osterberg.[5] Sutter et al stated that this interindividual variation of the cone density might be responsible for the interindividual amplitude variation of the multifocal ERG at the fovea. In our study, the responses by plural hexagonal stimuli were totaled and averaged, respectively, in all portions except the fovea, while the response of the fovea is the focal ERG itself extracted from a single hexagonal stimulus. So the response could be easily influenced by only a small noise, which may be one of the causes of the interindividual variation in the fovea. The latencies of N1-waves and P1-waves tended to be long in the fovea, become shorter in the parafova, and again longer in the periphery. Using his focal ERG system, Miyake[6] reported a similar finding. It is interesting, although the underlying physiologic mechanism remains unknown.

Table 4. Latency (msec) and Amplitude (nv) (Mean ± Standard Error) of the N- and P1-Waves at the Papillomacular Bundle and the Area Symmetrical to It (Areas 6 and 7)

	Area 6	Area 7
N1 latency	21.25 ± 0.43	21.00 ± 0.41
N1 amplitude	8.72 ± 1.04	7.28 ± 1.21
P1 latency	37.42 ± 1.26	37.10 ± 1.42
P1 amplitude	21.30 ± 1.88	21.53 ± 1.85

The amplitude was larger in the upper retina than in the lower retina. The N1 and P1 latencies were shorter in the upper retina than in the lower retina. A functional asymmetry between the upper retina and lower retina has been reported by focal ERG,[6,7] visual evoked potentials,[8] visual acuity,[9] and the standing potential of the eye.[10] These reports suggest a superior visual function in the upper hemiretina over the lower hemiretina. It is unknown from which portion of the visual tract this difference originates. Although, in the conventional ERG, the functional differences between the upper and lower retina could not be seen, Miyaki et al[6,7] reported that in the a-wave, the b-wave, and the oscillatory potential obtained by focal ERG, using 15° hemifield stimulation, the amplitude in the upper retina was slightly but statistically higher and that at least one part causing the functional asymmetry between the upper and lower retina originates in the photoreceptors. This agreed well with the asymmetry of the number of visual cells between the upper and lower retina obtained by Osterberg,[5] demonstrating that the ERG fields derived from the new technique of multifocal ERG indeed reflect the local function of the outer retina. In our study using the multifocal ERG, which is thought to reflect the response from the outer retinal layer as described above, the response from the upper retina was statistically larger than that from the lower retina.

The comparison of latency and amplitude between the nasal and temporal retina showed no statistical difference. Sutter et al[1] reported that asymmetry between the nasal and temporal areas could be seen in every subject and that higher response density could

be obtained in the nasal retina. This result by Sutter et al is in proportion to the cone density of human retinal areas reported by Curcio et al.[4] In our study, there was no statistical difference between the nasal and temporal areas. Furthermore, we found no statistical difference between the responses from the papillomacular bundle and those from the symmetrical temporal retinal area. The amplitude and latency of the P1-wave and the N1-wave showed no obvious correlation with a subject's age and refractive error in this study.

The advantages of the multifocal ERG are summarized as follows: (a) it enables quanitification of the limit and severity of retinal functional abnormalities, (b) it is an objective examination, and (c) the examination time is short. On the other hand, the disadvantages are summarized as follows: (a) it requires patient cooperation, (b) it uses a microelectropotential that is easily affected by ocular movement and eyelid movement, and (c) subjects are limited to those who can wear contact lenses and whose eyes can be dilated. Because of the factors related to the stimulation apparatus (reflection, the frame of the glasses, and so on), the reliability decreases in the peripheral region. Although there are some disadvantages, mentioned above, and the details of the origin of the response are yet unknown, the spatial property of multifocal ERG agrees well with the spatial distribution of the human retinal cones. A low amplitude corresponding to the optic disc can be detected. In clinical application for retinal diseases, the response corresponding to the damaged retinal area was diminished in age-related macular degeneration, as reported by Bearse et al.[11]

Multi-focal ERG would be useful for objective functional examination of the retina. In the future, clinical application of multifocal ERG for various retinal disorders is expected to reveal from which layer of the retinal structure the response is derived.

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